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Liver Lesions in Feral Fish: A Discussion of their Relationship to Environmental Pollutants

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NOTICE

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1. INTRODUCTION

The occurence of liver tumors in fish from polluted waters is becoming an issue of major concern among aquatic scientists and legislators. Survey studies of fish from highly contaminated areas have reported prevalences of lesions of up to 12%, 25%, or 33% of sampled populations. The carcinogenic nature of many contaminants, coupled with the known sensitivity of certain fish species to hepatotoxins, support the contention that pollution is playing a role in the development of the lesions. This contention will be reviewed and assessed in this report.

The implications of a relationship between liver lesions in fish and pollution are several. First, fish are increasingly recognized as effective "sentinel organisms" for environments at risk. The Status and Trends (S&T) pollution-monitoring program of the National Oceanic and Atmospheric Administration (NOAA) is using liver histopathology of selected fish species to monitor the environmental status of USA coastal waters. Although there is disagreement on the interpretation of data on the health of fish with regard to its reflection of environmental health, many studies have focused on fish as sentinels in order to better define and demonstrate this relationship.

Whether fish tumor epizootics can result in human health hazards for humans consuming diseased fish is unknown. Concern exists that human consumption of tissues of fish with liver tumors may result in ingestion of high concentrations of carcinogens--high enough to eventually induce similar tumors in the consumer. Again, this concern rests on the assumption that pollutants are causing the tumors. Scientists at the University of Washington School of Fisheries are now carrying out a human toxicology study to determine the level and the nature of toxic chemicals injested by the populations most at risk. The researchers are surveying Puget Sound subsistence fishing populations to assess the parts and quantities of fish tissues they consume.

The effect of liver neoplasia on the fish themselves is very poorly understood. Many of the liver growths in fish seem able to continue performing the vital functions of normal tissue (Hendricks, 1982, and Sinnhuber et al., 1968). Hendricks (1982) records observing "normally swimming and feeding trout in which the normal liver has been almost

totally replaced by large tumors." Murchelano (1985) reports examining healthy looking flounder in which up to 90% of the liver was grossly tumorous. Observers in Puget Sound have found no significant stunting of growth of English sole afflicted with hepatic neoplasms (L. Rhodes, pers. comm., 1985).

2. FIELD STUDIES

A connection between polluted aquatic environments and hepatic liver lesions in fish was first suggested by anecdotal field surveys. Scientists sampling species in polluted waters would come across surprising numbers of fish with lesions (Dawe et al., 1954; Baumann et al., 1982; Smith et al., 1979). This led them to suggest that environmental pollutants were inducing the observed tumor epizootics.

As interest in the problem increased, field studies became more epidemiologically and histologically thorough, thus providing evidence for the original suggestion. A handful of convincing reports of tumor epizootics in feral fish populations have now accumulated.

Although they differ widely in their geographic and ecological locations, all studies have two things in common: the type of fish examined and the quality of the environment studied. This fact in itself is evidence for a lesion-pollution relationship.

In marine areas, sole and flounder afflicted with tumors have been found (Malins et al., 1984 and Murchelano and Wolke, 1985). In freshwater, "rough" fish--bullheads, carp, sauger--are the fish involved in the epizootics (Baumann et al., 1982; Brown et al., 1973; Black et al., 1982). All of these species are bottom-dwelling and bottom-feeding and have relatively limited migratory ranges (Black, 1983; Malins et al., 1983; W. Bridges, pers. comm., 1985). They are thus likely to be exposed to polluted environments for periods long enough for tumors to develop (Baumann et al., 1982; Brown et al., 1982).

The deteriorated quality of the environment in which these fish live is the other common element in all field studies to date. Sites of epizootics are typically polluted with complex chemical and industrial wastes. PAHs especially have been found in abundance in the sediments of a number of study sites. No studies sampling in unpolluted bodies of water have ever discovered elevated incidences of hepatic lesions in the fish. The number of these studies is, however, limited. Increased and widespread monitoring of both clean and polluted sites is still necessary for the establishment of a stronger relationship.

The body of this paper discusses several relevant field studies done between 1964 and 1985 with respect to hepatic tumors in fish samples and discusses their methods and their strengths and weaknesses. Recommendations for further study and procedures are also given.

2.1. Dawe et al., 1964

Dawe et al. (1964) were the first to report the presence of hepatic tumors in a population of feral fish. They identify the neoplasms in

fish collected during a state survey of Deep Creek Lake, in Maryland. One of two brown bullheads (Ictalurus nebulosus) and three of twelve white suckers (Catostomus commersoni) bore the lesions. The diseased white suckers were also affected by what was thought to be a hepatic parasite, which could not be convincingly associated with the neoplasms. All fish carrying tumors were among the oldest in their respective population.

Dawe et al. conjectured that some environmental factors had played a role in inducing the observed lesions. Deep Creek Lake was known to be polluted by hydrocarbons from motorboat exhaust, by pesticides, and by rotenone; these compounds could have accumulated in the lake's sediments. Long-term contact with the sediments by the suckers and bullheads--both bottom-dwelling fish--could have resulted in tumor induction by any one of the compounds. The Dawe et al. study, however, was anecdotal, and these etiological conjectures were not supported by any evidence. In their paper, the researchers felt it "decidedly presumptive to consider an environmental carcinogen combined with a high species susceptibility as explanations for the findings" (p. 1196). Subsequent studies and finds, however, made their statement lose much of its "presumptive" nature.

2.2. Smith et al., 1979

The Smith et al. survey study of the Hudson River Estuary (1979) reported a high prevalence of hepatic lesions in fish from this area. Of 264 Atlantic tomcod (Microgadus tomcod) collected in the estuary, 25% suffered from lesions defined as "hepatomas" with different degrees of severity (p. 314): 66 fish showed "neoplastic nodules of hepatocytes hepatocellular cinoma" (p. 314). In addition, 43% of the fish had other types of less serious lesions. Smith et al. blamed high tissue concentrations of PCBs (10.9-98.2 ppm in livers of fish with lesions, vs. 20.3-49.2 ppm in livers of fish without) for the pathologic damage observed. PCBs were also found in elevated concentrations in the Estuary sediments. The concentrations of other contaminants in the tomcod tissues were still being determined at the time of publication.

The arguments of Smith et al.for the role of PCBs in tumor formation in the tomcod are weak; their fallacies include their disregard of etiological factors besides these contaminants. The researchers emphasize that PCBs have been shown to be carcinogenic in mammals and that these compounds have been found in other areas of tumor epizootics. However, PCB carcinogenicity in fish has never been demonstrated (C. Dawe, pers. comm., 1985).

Moreover, Smith et al. lack an epidemiologic assessment or a control to reinforce their argument. Their study, however, does bring to light the existence of an area of concern, and a point of departure for future evidence concerning the role of pollutants on hepatic tumor induction.

2.3. Black et al., 1982

A 1982 study by Black et al. is also little more than an anecdotal account. It reported that 100% of 23 sauger (Stizostedion canadense) collected from a copper-polluted Michigan lake bore liver lesions. Twenty fish obtained in 1979 and three in 1980 had hepatic nodules diagnosed as neoplasms. The sizes and numbers of the nodules varied with the specimen. Three out of eleven walleyes (Stizostedion vitreum) caught from the lake were also affected by hepatocellar carcinoma. Small numbers of other species of fish collected (northern pike, white sucker, smallmouth bass, rainbow and brook trout) showed no evidence of tumors.

The watershed where these fish lived, Torch Lake, had been used for years as a repository of copper mining wastes. The range of levels of this metal was 30-40 ug/l in the water and 1700-2400 mg/l in the upper flocculent sediments at the time of the study. Copper was the sole serious pollutant in the lake, and Black et al. attributed the high incidence of neoplasms in the sauger to the presence of the copper. Since copper per se has never been shown to be carcinogenic, the authors discussed alternative pathways by which the pollutant could have caused the observed lesions. They referenced a study linking copper-caused cirrhosis and liver tumor occurrence in humans. None of the fish they observed, however, had cirrhotic livers, and although they postulated the possible carcinogenic role of the extremely fine copper tailings, the authors could offer no actual evidence of the hepatic toxicity. Black et al. suggested, after Croisy et al. (1980), that the copper might be catalyzing the formation of carcinogens (nitrosamines) from more innocuous nitrogen wastes in the lake. They did not, however, measure the levels of such potential pollutants. More information on the type and concentrations of organic compounds in the lake would have strengthened this study.

The Black et al. discussion of the possible etiology of the lesions included a reference to the age of the fish involved: an average of eleven years for all saugers collected during the study. It is reasonable to suppose that liver damage in fish of this age is normal. Given the absence of information about the state of saugers in other nonpolluted lakes, this point remains unresolved. A control site and a consideration of alternative etiologies would have improved the quality of this study.

2.4. Malins et al., 1984

The most convincing field evidence for the relationship between pollutants and hepatic neoplasms in fish stems from the vast amount of work done in Puget Sound by Malins and his collaborators. Through a four-year study (Malins et al., 1984), this research group correlated levels of contaminants in the sediments of the Puget Sound with prevalence of liver lesions in resident demersal fish species (English sole, Parophrys vetulus, rock sole, Lepidopsetta bilineata, and Pacific staghorn sculpin, Leptocottus armatus). The investigators analyzed the content of the sediments and the status of the tissues of fish from various sites in Puget Sound. Statistical analysis of their results

showed that the sites most contaminated with aromatic hydrocarbons (PAHs) and metals--but not PCBs--hosted the highest percentages of fish with liver neoplasms. The most severely affected species, English sole, suffered a 12.1% prevalence of neoplasms at the most highly polluted site.

A role for PAHs in tumor formation had been suggested by Malins et al. in earlier work (Malins et al., 1983): high concentrations of PAH-derived free radicals were observed in livers of fish carrying tumors. Malins et al. (1984) also suggest that metals might cause changes in the levels of compounds that provide defense against carcinogens in fish livers, but the link between metals and lesions has less support.

The lesion prevalences reported by Malins et al. now need to be age- and sex-normalized. Many pathologies increase in prevalence and severity in older fish, and a particular sex may be more sensitive to certain diseases. It is, therefore, important that lesion prevalences at different sampling sites be compared among corresponding groups of fish. Moreover, Malins et al. must obtain more thorough knowledge of the migratory behavior of the fish they studied. Such knowledge will help determine the relationship between a fish's site of tumor induction and the site of its capture.

2.5. Landolt et al., 1985

Malins et al., 1984, also contend that specific pollution "hot spots" in Puget Sound foster the production of lesions in fish. This theory is supported by a recent report by Landolt et al. (1985). Whose study assesses the baseline condition of fish from a pristine area of Puget Sound. Over the course of two years, these authors collected 1,468 fish--English sole among them--from three sampling sites in the area. After examining the fish for abnormalities (using the same histologic critera as Malins et al.), the authors reported the fish's health as "excellent," and found "no frank neoplasms," hepatic or otherwise.

Results of Landolt et al. support the contention that fish living in polluted sites are more likely than fish in pristine waters to develop tumors. Before this can unquestionably be concluded, however, the results must be age- and sex-adjusted, and the migratory pattern of the fish better clarified.

2.6 Murchelano and Wolke, 1985

The Puget Sound tumor epizootic is unique in having been extensively studied. Other regions with high hepatic tumor prevalences in fish are just beginning to receive attention. A high prevalence of liver cancer in winter flounder (Pseudopleuronectes americanus) from Boston Harbor was just recently reported. Murchelano and Wolke (1985) present a histologic discussion of the hepatic lesions observed in the liver of both winter flounder and windowpane flounder (Scophthalmus aquosus) at several sites along the USA northeast coast.

Samples included 319 fish from polluted sites and 93 from control areas collected from sampling sites in Connecticut, Rhode Island, Maine, and Massachussetts. Of the flounders from the polluted sites of New Haven Harbor and Narrangansett Bay, 10% had preneoplastic lesions, 3.4% had neoplastic lesions. Of the fish from Boston Harbor, 7.5% had actual neoplasms. Fish from the control areas off Long Island and Maine showed no signs of lesions.

The investigators focused on the histology of the lesions observed in their survey. They concluded that the tissue damage in the Massachusetts fish was "consistent with the action of a hepatotoxin" (p. 589). They therefore used histologic evidence to implicate pollutants in the lesion-causing process. They did not discuss contamination levels at their sampling sites, but Boston Harbor is known to contain pockets of high PAH levels (5), as well as PCBs and heavy metals (9).

Murchelano and Wolke's is only an initial, descriptive report of a tumor epizootic. Stronger epidemological research, more exhaustive sampling, and complementary laboratory work will provide clearer understanding of the lesions' etiology.

2.7. Baumann et al., 1982

Baumann et al. (1982) undertook a study to assess the role of industrial contaminants in the formation of tumors in fish. Their strategy was to compare prevalences of hepatic lesions in brown bullhead (Ictalurus nebulosus) from polluted and nonpolluted freshwater sites. Their chosen contaminated site was the Black River in Ohio. Its sediments were measured to contain PAH levels of up to 390 ppm; the levels of other contaminants were not recorded. The reference site, Buckeye Lake, was known to be free of industrial wastes. Analyses for PAHS in its sediments were still ongoing at the time of publication.

Baumann et al. recorded a 33% prevalence of hepatic tumors among Black River bullheads three years or older. They found no tumors in the bullheads from the control site. These researchers suggest that the PAHs were "the most likely causal factor" (p. 100) for the tumors found in fish from the Black River.

The work of Baumann et al. contains experimental omissions and inconsistencies that undermine its credibility. For example, it lacks both a clear identification of the fish-sampling sites in the river and a presentation of their corresponding sediment PAH levels. The study reports contaminant concentrations from just one site without specifiying whether any fish were caught there. The number of fish collected in the study is itself unclear. From the standpoint of pathology, the report describes the tumors in little detail, not enough to provide a clear representation of the lesions observed. Etiologically, the report fails to consider any variable besides PAHs which could contribute to tumor formation. It also lacks a discussion of river parameters--such as

sediment types or water flow--which might influence exposure of the fish to the suggested causal agent. A more thorough, rigorous study would provide much stronger evidence for the link between PAHs and liver tumors that the authors suggest.

2.8. Brown et al., 1973

Brown et al. (1973) conducted a five-year survey of the neoplasms found in fish from the polluted Fox River watershed in Wisconsin. The river water contained chlorinated hydrocarbons (0.21 ppm), naphthalene (0.1 ppm), and toluidine (0.2 ppm), as well as other industrial contaminants. Coliform counts higher than 30,000/100 ml water were recorded in the river for the months of June to October.

The control site for the study was the Lake-of-the-Woods, in Canada, in which "no indications of chemical pollutants or of fecal bacterial presence" (p. 191) were found. Brown et al. attest that both reference and study sites had comparable physical and biological variables, including current flow, water depth and temperature, and resident fish species. Nonetheless, one could remain skeptical of the choice of a lake as a control site for a river.

When the types and numbers of neoplasms in fish from the two areas were compared, consistently more abnormalities were recorded from the polluted river. Brown bullheads were the fish most prone to carrying tumors. Of 283 specimens collected from the river over the five-year study period, 35 (12.21%) showed evidence of neoplasms, with "hepatoma" (p. 196) as the most prevalent. Only a 1.98% hepatoma occurrence (2 of 101 fish) was noted in the control lake.

The researchers argued in their discussion that the pollution in the Fox River had "enhance(d) carcinogenesis" (p. 195). Their conclusion contained some etiological considerations: they noted no differences in tumor prevalence among fish with parasites or mechanical damage. They did not, however, screen the diseased fish for viruses. They did report an increase in the tumor incidences during the times of the year when the coliform counts in the river were highest. This correlation could suggest a pathogen-related etiology for the lesions observed. However, given the time lag usually necessary for tumor development, the relation may be simply coincidential. Because of the lack of pathologic and histologic details on the lesions, this point cannot be determined.

This study's main shortcoming is the incompleteness of the description of the pathology. The information provided permits no establishment of the nature or severity of the tumors, and it remains impossible to determine whether they could have resulted from the action of a hepatotoxin.

2.9. Sloof, 1983

Sloof's 1983 study on tumor incidences in the Rhine River Basin argues for the inadequacy of using fish lesions as indicators of contaminated environments. Sloof examined a total of 7,737 fish from the

different rivers and lakes in the Rhine Basin. The Rhine, the most polluted of the rivers sampled, contains benzo-a-pyrene, beuzofluoranthene, and benzene at levels of 0.1-1 ug/L, and carbon tetrachloride and other chlorinated hydrocarbons in 1-10 ug/L quantities.

Only 8 of all the fish caught were observed to be affected by neoplastic liver lesions. All were bream (Abramus brema). Potentially neoplastic lesions such as "chronic inflammation," "hemorrage and necrosis," or "focal areas of non-eosinophilic parenchymatic cells" were found in 813 other miscellaneous species samples. The neoplastic potential of these lesions is unclear. The total prevalence of hepatic tumors for bream in the Rhine River area was approximately 0.1%. No tumors were observed in fish caught at the control site, Lake Braaseur.

The low prevalence value contrasts with the much higher ones recorded in hepatic lesion epizootics. Yet Sloof described the bream as a "homebound bottom feeder," able to metabolize contaminants to reactive species (Sloof and Van Kreijl, 1982). It remains to be extablished, however, whether bream are indeed injesting or otherwise coming in contact with the river's carcinogens. The fish's pathologic sensitivity to the reactive metabolites is also uncertain.

Sloof's paper is a demonstration that water pollution alone does not necessarily cause tumor formation. Sloof suggests that factors in addition to chemical pollutants must be involved in the induction of neoplasms in fish, but that such factors are not at play in all instances. Reaching some appreciation for what these factors are and how they can act could lead to the pinpointing of areas and species more vulnerable to cancer.

3. EPIDEMIOLOGY

The studies summarized above provide good circumstantial evidence for the liver tumor-pollution relationship. None of them, however, is rigorous enough to stand alone as proof of it; none tries to. The difficulties inherent in establishing rigorous experimental conditions in the wild prevent field studies from ever presenting irrefutable evidence. Nonetheless, many of the studies could acquire greater validity were they to consider certain essential epidemiological factors.

The age and sex of a fish may affect the likelihood of its carrying a tumor; its dietary habits and migratory patterns can determine its degree of exposure to pollutants; the physical qualities of its habitat --sediment type, current flow--will play a role in pollutant bioavailability. In an ideal field study, all these confounding factors would be considered when comparing tumor incidences at different sampling sites.

The need for age adjustment of liver tumor prevalences has been clearly demonstrated by both laboratory and field studies: older fish are generally more likely to have tumors. Bullheads from the Black River, Ohio (Baumann et al., 1982), for example showed a 33% hepatic lesion

incidence in specimens older than three years, as opposed to 1.2% in two-year-olds. In Torch Lake, Michigan, where the incidence of hepatocarcinoma was 100% among the captured sauger, the fish observed averaged age 11 years (Black et al., 1982). These trends may be due to the decrease with age in the immunological capability of fish. (Good and Finstad, 1969). Alternatively, older fish may have had prolonged exposures to carcinogens or an increased lag time after exposure, and this have become more likely to develop tumors. It is, therefore, important to compare tumor prevalences at different sites only among the same age groups.

The same reasoning applies when the sex of the fish is considered. Female zebra danios (Danio rerio) were shown to be more sensitive to the tumorigenic effect of nitrosamines than males (Matsushima and Sugimura, 1976). DDT fed to female trout yielded a 74% tumor induction as oppposed to 21% in males (Hendricks, 1982). These types of sex-dependent susceptibilities should be considered when prevalences of hepatic tumors are compared.

Field-sampling areas from which prevalence data is obtained must likewise be comparable. The control site selected in a field study should approximate the actual study area in its physical and biological characteristics and should vary only in pollution levels. In practice, such a clean site is extremely difficult to locate. It is sometimes impossible even to find a reference site pristine enough to determine the natural tumor prevalence of the fish species under study. In such cases, an idea of the variability of prevalences may be obtained by sampling multiple sites of varying sediment pollutant concentrations (L. Rhodes, pers. comm., 1985).

A fish's migratory pattern must also be considered in field studies. Migration will determine whether a fish is caught in the same area in which its tumor was induced (A. Mearns, pers. comm., 1985). For wide-ranging fish, correlation of location at time of capture with tumor induction at that site may be meaningless. For homebound species, the relationship may hold: winter flounder in Boston Harbor, for example, were shown through a tagging study note to stray beyond a 1-mile area (Bridges, 1985).

4. CHEMICAL CARCINOGENESIS

The contention raised by field studies that pollution may be involved in fish tumorigenesis is supported by laboratory evidence. Experimental studies have shown the susceptibility of livers of certain fish species to carcinogenic induction by chemicals. Such studies, coupled with the demonstrated carcinogenicity of certain marine pollutants, argue for a link between the pollutants and the hepatic lesions of feral fish.

The sensitivity of fish to chemical carcinogens first became apparent in the 1960s, when aflatoxin--a tumorigenic compound produced by certain species of molds--was recognized as the cause for hepatic

growths in hatchery rainbow trout (Salmo gairdneri) (Ashley and Haluer, 1961). This species has since been established as the organism most sensitive to tumor induction by this carcinogen (Sinnhuber et al., 1977). Exposure to aflatoxin B1 (AFB1) through either diet or surrounding water is effective in inducing tumors (Sinnhuber et al., 1977). Dietary administration of 4 ppb AFB1 to the trout over a period of 1-30 days, followed by a one-year lag time, can produce a tumor incidence of between 10 and 40%. Levels of AFB1 as low as 0.4-0.5 ppb, continuously fed in the diet, can also induce tumors. Daily 1-hour exposures of trout embryos to a solution of 0.4 ppm AFB1 yield hepatic tumors after 10-12 months (Henricks, 1982). Even a brief, one-time immersion of the eggs in 0.5 ppm AFB1 can initiate hepatic tumor growth in 6-12 months (Hendricks, 1982). For either the dietary or the integumental route of intake, as the time after exposure increases, so does the percentage of fish with tumors in the experimental population.

This last result also holds true for other categories of mammalian carcinogens. Nitrosamines, azobenzenes, thiourea, carbon tetrachloride, and DDT are some of the compounds that have been shown to be liver carcinogens in the trout (Hendricks, 1982). None, however, was effective at concentrations as low as those used with aflatoxin. DDT was fed at 25 ppm for 21 months, at which time 52% of the trout in the experiments had developed liver tumors. Nitrosamines induced tumors in the trout as well as in other fish species: N-nitrosodiethylamine and N-nitrosodimethylamine dissolved in water proved carcinogenic at doses ranging from 32.5-100 ppm in guppies (Poecilia reticulata) and from 10 to 100 ppm in zebra danios (Danio rerio) (Matsushima and Sugimura, 1976).

In the laboratory, the sensitivity of certain species of small fish to hepatotoxins has resulted in their becoming increasingly important in carcinogen testing. In general, the fish's short response time, low cost, and easy upkeep make them attractive alternatives to laboratory mammals. Their experimental use has permitted the accumulations of a large body of knowledge on the process of liver carcinogenesis in fish. The effectivess of even a single carcinogen exposure for tumor induction and the need for a reasonable time lapse between exposure and subsequent tumor development are two generally applicable principles stemming from this knowledge. They must be considered whenever the tumorigenic action of pollutants in the marine environment is being assessed.

4.1 PAHs and Carcinogenicity in Fish

Laboratory work that has given direct evidence to support field observations has focused on the carcinogenic effects of PAHs, polynuclear aromatic hydrocarbons. PAHs are the chemicals that have been inculpated most frequently with causing the neoplastic lesions seen in marine organisms. PAHs are ubiquitous in the marine environment, but they are found in abundance in urban and industrialized areas (C. Dawe, pers. comm., 1985). Their high concentration in the sediments in areas of fish tumor epizootics (Baumann et al., 1982; Brown et al., 1973; Malins et al., 1984) coupled with their demonstrated hepatic carcinogenicity in mammalian systems (Siegel et al., 1973) strongly point to their role in lesion induction in the feral fish. Laboratory experiments are now establishing the ability of PAHs to produce neoplasms in fish.

Schultz and Schultz (1982) showed 7,12 dimethylbenzanthracene (DMBA) (a derivative of benzanthracene, one of the most common PAHs found in the environment) to be carcinogenic in two different species of aquarium guppies, Poeciliopsis lucida and Poeciliopsis monacha. Both young (1-30 days) and older (2-7 months) specimens were used in the experiments. No tumors were induced in the young fish upon their exposure to 0.25 ppm DMBA in water for two to four 20-h periods (a higher dosage of 0.5 ppm had proved toxic). However, doses administered to older fish did elicit tumorigenic response: 5 ppm DMBA present in the aquarium water for three to four 20-hr periods produced liver tumors in 22 of the 46 older fish surviving the seven to eight months lag time.

Schultz and Schultz's experiments contradict earlier work by Pliss and Khudoley (1975): the latter had demonstrated the inefficacy of DMBA and 3-methylcholanthrene (3-MC, another PAH) in causing tumor development in fish. The researchers had administered the PAHs to guppies (Poeciliopsis reticulata) and zebra fish (Danio rerio) by a variety of protocols: by intraperitoneal and intramuscular (im) injection (40 mg DMBA, 20 mg 3-MC lagtime of 80 weeks); by intraperitoneal pellet implantation (1 mg DMBA lagtime of 32 weeks); by skin application (0.2-0.3% in acetone solution lagtime of 28 weeks); and by feeding (120-mg/100-g dry diet, 18 weeks lagtime). None of these methods induced tumor growth, although most of the fish were dead by the end of the observation period. Schultz and Schultz (1982) suggest that the "inadequate concentrations of DMBA reaching susceptible tissues" were responsible for Pliss and Khudoley's (1975) negative results.

The differences in results in the two experiments emphasize the influence that the route of administration, the age of the fish, or the dosage used might have on the carcinogenic effect of a given compound. These factors must also to be taken into account in order to assess a pollutant's role in a cancer epizootic in feral fish.

Actual PAH mixtures extracted from polluted field sediments are now being tested for their carcinogenic potential. They are being administered to laboratory-kept fish in dosages and combinations approximating field conditions. The experimental assessment of their carcinogenicity will yield information extremely relevant to understanding the etiology of lesions seen in wild fish.

Black (1982) used a sediment extract of PAHs to test his contention that the compounds were responsible for inducing dermal neoplasms in feral bottomfish. He extracted the contaminant mixture from Buffalo River sediments and administered into the skin of brown bullheads (Iictalurus nebulosus), which are bottom-feeding fish, likely to experience the effects of prolonged exposure to contaminants in the field.

The experimental extract was concentrated from an initial 76 ppm of PAH in the sediments to a 5% wt/vol solution and then was applied to the upper lip of the fish once a week. A gradual transformation of the

treated skin to papillomas occurred within a 12 to 14 months. No changes were seen on the skin of bullheads treated only with the extracting solvent. Black used this contrast as evidence of the potentially tumorigenic nature of the contents of the polluted river sediment. However, he did not try administering "clean" sediment extracts to the fish skin. A study using this control procedure is necessary to strengthen his contention.

In Seattle, Malins' group recently began an experiment to test the liver carcinogenicity of polluted sediments in fish (Harden, 1985). Malins et al. injected extracts from Puget Sound sediments in to English sole (Parophyrys vetulus) kept in laboratory tanks. The extracts contained mainly PAHs and nitrogen heterocycles. Their effect on the fish will be gauged after a determinate time lag.

Individual, purified PAHs and nitrogen compounds known to be contained in the sediment extracts were also administered to different groups of sole. Their specific effect on the fish will be compared with that of the extract, which will permit the identification of the most hepatotoxic components of the sediments and will establish the occurrence of any synergistic or antagonistic effect of the chemicals.

Malins' experiment is a good approximation of a controlled field study: it utilizes the fish species found to bear tumors in polluted areas and the chemicals found in those areas. However, it needs to use methods of contaminant administration other than injection to more closely simulate field conditions.

4.2 MFOs and Carcinogenicity

A further line of evidence for the carcinogenicity of PAHs in fish comes from an understanding of the physiologic processes leading to tumor formation. PAHs, like many other compounds, must be metabolically activated before they act as carcinogens (Stegeman et al., 1982). Activation is provided by specific hepatic enzymes known as mixed function oxidases (MFOs); these enzymes are reponsible for oxidizing foreign compounds to more soluble, and hence more readily excretable compounds. In doing so, they can transform relatively inert molecules into very reactive products, some of which are capable of tumorigenic damage (Sims and Grover, 1974). Different MFOs will yield products with smaller or greater carcinogenic potential: the specific battery of metabolizing enzymes present in an animal's liver can therefore determine its susceptibility to carcinogenesis (Stegeman, 1981; Stegeman et al., 1982; Stegeman et al., 1984). The presence of hepatic enzymes capable of producing deleterious metabolites in feral fish is thus additional evidence for the ability of pollutants to induce tumors in these organisms.

The production of reactive metabolites is only the first in the series of biochemical events that will lead to tumorigenesis. The link between the initial and final event is still tenuous. It is nonetheless being supported by a growing amount of evidence. Varanasi and Gmur (1980) compare the metabolism of benzo-a-pyrene (BaP) to potentially

carcinogenic species by English sole (Parophyrus vetulus) and starry flounder (Platichtys stellatus). Both fish showed a very thorough (85%) biotransformation of the BaP to metabolic products, but the English sole showed itself to be the more extensive metabolizer and the one to form a greater proportion of polyhydroxy species--reactive intermediates with carcinogenic potential. The researchers correlated these findings with prevalences of liver tumors higher in English soles than in starry flounders seen in waters polluted with PAHs.

Another study linking the production of reactive metabolites with tumor development was performed on English sole from the Duwamish River (Malins et al., 1983). Malins et al. measured the concentration of free radicals present in the livers of lesion-bearing and healthy sole. Organic free radicals are very reactive chemical species which can be formed during xenobiotic metabolism. They can be involved in generating lesions leading to cancers.

Diseased fish were shown to contain higher concentrations and different types of organic free radicals than the ones not bearing tumors. In other words, a higher free radical content corresponded to a higher probability of liver lesion presence. When PAH extracts from the sediments of the Duwamish River were incubated with liver enzymes from normal sole, free radicals were generated (Malins et al., 1983). However, when the PCB, alkane, or polar fractions extracted from the river were used as substrates, no free radicals were detected. These results demonstrate the ability of the sole to form potentially tumorigenic products from some environmental pollutants, and they strengthen the case for PAHs as an effective carcinogen in contaminated sediments.

5. ALTERNATIVE ETIOLOGIES

Most field and laboratory studies on fish liver lesions have been aimed at demonstrating the role of pollutants in tumor formation. Few other causative agents have been rigorously discussed or tested. Yet, the etiology of hepatic neoplasia in fish is so poorly understood that no study should champion a particular causal factor without considering, or at least discussing, possible alternatives.

Viruses have repeatedly been suggested as alternative carcinogenic agents in the liver tumor epizootics (A. Mearns, pers. comm., 1985; R. Sonstegard, pers. comm., 1985; H. Stick, pers. comm., 1985). Evidence for the ability of these agents to produce liver lesions comes from work in mammalian systems: in humans, a relationship between viral hepatitis B and hepatocellular carcinoma (HCC) has definitely been shown to exist (Edmonson and Peters, 1982). High percentages of patients suffering from the viral infection eventually develop liver neoplasms. This is true for as many as 80% of all hepatitis B cases in some regions of Asia and Africa.

Evidence for the ability of viruses to cause lesions in marine organisms is available for certain types of growths. Some lymphosarcomas

and skin papillomas, for example, are thought to be induced by viral agents (Wolf, 1972), although no hard proof of this exists (C. Dawe, pers. comm., 1985). No studies have yet adequately attempted to demonstrate a viral etiology for hepatic neoplasms in fish (R. Sonstegard, pers. comm., 1985; H. Stick, pers. comm., 1985). Electron-microscopical examination of neoplastic liver tissue of Boston Harbor winter flounder, however, has so far revealed no evidence of viruses (R. Murchelano, pers. comm., 1985). There is also no known example of viral hepatitis in fishes (C. Dawe, pers. comm., 1985).

It has been hypothesized that a viral agent in conjunction with the influence of contaminants might be causing the observed liver tumors in fish (R. Sonstegard, pers. comm., 1985). The presumptive agents could conceivably synergize with each other by affecting different aspects of the tumorigenic mechanism (Casto and D. Paolo, 1973; Mahdy and Dukta, 1973; Sonstegard, 1977). The pollutants might impair the antiviral immunological competence of an organism, for example; or they might render cells more susceptible to viral infections or they might activate latent oncogenic viruses. Viruses, on the other hand, might also impair an organism's immune response and render it more susceptible to chemical carcinogens; they might alter its detoxifying system and thus lead to the increased or decreased production of metabolites; they might increase the permeability of certain cells, allowing easier entry of the chemical carcinogens.

Whether any of the scenarios described above are at play in the case of hepatic tumors in fish is speculative. Clearer proof for the involvement of a virus in the neoplastic process must be provided before any supportive role of pollution is invoked. It is unfortunately virtually impossible to prove the absence of viral involvement (C. Dawe, pers. comm., 1985).

Factors besides viruses might also be responsible for making certain fish populations hypersusceptible to tumor induction. An epizootic of hepatic lesions in a given fish population might be due more to the predisposition of the species than to an excessive level of contaminants --exposure to pollution alone does not necessarily lead to tumors formation. Sloof (1983) reported that only 3 out of more than 4,000 fish collected from the polluted Rhine River had liver lesions; Sonstegard (pers. comm., 1985) found only occasional epizootics of fish lesions during decades of sampling in the contaminated Great Lakes. Genetic, biochemical, or physiological factors, unique in each species of fish, may enhance or reduce its susceptibility to carcinogens.

The predisposition of certain genetic strains or species to tumorigenesis is well recognized (Mawdesley-Thomas, 1972). This predisposition might result from the presence of particular metabolic enzymes or from immunological deficiencies. A genetically based malfunction of an organism's immune system could result in an altered capability of dealing with carcinogens (Good and Finstad, 1969; Mawdesley-Thomas, 1972): an organism's ability to eliminate neoplastic cells might be reduced, making it more susceptible to tumor formation.

Such a malfunction might explain the higher hepatic tumor prevalence seen in older fish: involution of the lymphatic system observed with increasing age might result in decreased immunocompetence. However, no studies on the relationship between immune response and tumor rejection in fish have ever been carried out (C. Dawe, pers. comm., 1985). Experiments testing for lymphatic malfunctions in fish affected with hepatic neoplasms would offer further support for this etiological possibility.

6. FUTURE RESEARCH DIRECTIONS

The carcinogenic action of contaminants on fish still remains the most likely etiology for hepatic tumors. Further research is now necessary to prove this, and many investigators agree that pivotal evidence is likely to derive from the use of feral fish in laboratory experiments.

In such experiments, fish known to acquire lesions in the wild could be maintained in laboratory tanks and subjected to contact with environmental concentrations of contaminants over their lifetime. Development of tumors could then be monitored and recorded (C. Dawe, pers. comm., Malins et al., 1984; R. Murchelano, pers. comm., 1985; R. Sonstegard, pers. comm., 1985). Such experiments would permit the effect of the environmental pollutants on the fish to be gauged apart from other factors.

The importance of the information that this type of experiment can provide has now spurred scientists to overcome the logistical problems. Malin's group has recently undertaken such an experiment with English sole (Parophrys vetulus) (Harden, 1985).

Keeping feral fish in controlled environments might also help answer other pertinent questions on fish tumors. Monitoring for the effect of the lesions on the fish's viability, longevity, and ability to reproduce could be done in laboratory facilities and could help determine the significance of the disease in terms of population effects or stock decline in the wild.

Shorter term experiments on hepatic lesions could yield physiological and etiological information. Physiological observations on the workings of the immune system of fish might determine whether the immune system is being impaired by contaminants and whether its disfunction alone (or in concert with carcinogens) leads to tumor induction (C. Reinish, pers. comm., 1985). Biochemistry could yield clarification of the processes of intake and transformation of PAHs by the fish (C. Dawe, pers. comm., 1985). Information of these types would increase the appreciation of the areas and fish most at risk and of the least damaging types of pollutants.

The etiological role of viruses also needs to be better established (R. Sonstegard, pers. comm., 1985). Experimental detection of these pathogens in tumor-bearing fish should be thoroughly attempted, through viral transmission or DNA hybridization studies.

New labwork must be paralleled by continued fieldwork. Not enough sites and not enough fish have yet been sampled (C. Dawe, pers. comm., 1985). New field studies would benefit from being designed in such a way as to be more epidemiologically rigorous and more comparable. The use of standardized survey methods and uniform processing of collected tissues is strongly recommended. Surveys of clean areas should be performed to yield knowledge on the background prevalence of hepatic tumors. In situ experiments in which cages containing a chosen fish species are hung in polluted waterways for specific lengths of time are suggested as alternative fieldwork approaches (Consensus Committee, 1984). Such experiments would provide information on the lengths of time necessary for tumor induction, and they could also gauge the role of polluted waters as opposed to sediments or food in the carcinogenic process.

Standardization of histologic terminology for observed lesions would also be an important achievement. A careful recording of a lesions' appearance would permit reliable comparison of findings at different study sites. Similarities in both fish lesions and environmental conditions from different sites could serve as evidence for the causal role of pollutants in tumor formation.

]7. REFERENCES

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